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A Novel G Protein α Subunit Containing Atypical Guanine Nucleotide-binding Domains Is Differentially Expressed in a Molluscan Nervous System*

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We describe the characterization of a novel G protein α subunit, $G\alpha_a$. cDNA encoding this subunit was cloned from the central nervous system of the mollusc *Lymnaea stagnalis*. The deduced protein contains all characteristic guanine nucleotide-binding domains of Gα subunits but shares only a limited degree of overall sequence identity with known subtypes (~30%). Moreover, two of the nucleotide-binding domains exhibit salient deviations from corresponding sequences in other G protein α subunits. The A domain, determining kinetic features of the GTPase cycle, contains a markedly unique amino acid sequence (ILIIGGPGAGK). In addition, the C domain is also clearly distinct (DVAGQRSL). The presence of a leucine in this motif, instead of glutamic acid, has important implications for hypotheses concerning the GTPase mechanism. In contrast to other Gα subtypes, $G\alpha_a$ has no appropriate N-terminal residues that could be acylated. It does contain the strictly conserved arginine residue that serves as a cholera toxin substrate in $G\alpha_s$ and $G\alpha_t$ but lacks a site for ADP-ribosylation by pertussis toxin. *In situ* hybridization experiments indicate that $G\alpha_a$-encoding mRNA is expressed in a limited subpopulation of neurons within the *Lymnaea* brain. These data suggest that $G\alpha_a$ defines a separate class of G proteins with cell type-specific functions.

Heterotrimeric G proteins (Gαβγ) form a family of molecular go-betweens that couple stimulus-triggered cell surface receptors to response-generating effectors within the cell (1–3). In reconstitution systems, specific G protein subtypes can interact with more than one receptor or effector subtype (1). Such a promiscuity might, in fact, allow the integration or distribution of extracellular signals *in vivo* (4). To address this issue, one needs to study intact cells since their complex plasma membrane organization furnishes a specificity of signaling that is significantly higher than that of reconstituted systems (5). Moreover, since signaling pathways may differ considerably in different cells, it is crucial to work with unambiguously identified cell types.

One system offering such opportunities is the simple central nervous system of the pond snail, *Lymnaea stagnalis*, which we use to study the function of G protein-mediated signaling networks in neuronal information processing. The *Lymnaea* brain contains large neurons that can be reproducibly identified from animal to animal, manipulated *in situ*, and cultured and studied *in vitro* (6, 7). Previously, we have cloned a diverse set of snail Gα subunits, *i.e.* $G\alpha_o$, $G\alpha_i$, $G\alpha_s$, and $G\alpha_q$, as well as a Gβ subunit (8–10). All of these *Lymnaea* subunits appear to be remarkably similar to their mammalian counterparts (76–82% amino acid sequence identity). Taking into consideration such a striking resemblance and the fact that at least 16 Gα subtypes exist in mammals (forming four classes), it seemed reasonable to assume that *Lymnaea* expresses more than merely four G protein α subunits (belonging to three classes).

Here, we describe the cloning of a novel Gα subtype, $G\alpha_a$, which has hitherto not been reported in any other species. Although clearly a G protein α subunit, it is unlike subtypes described to date. Two of its nucleotide-binding domains deviate markedly from analogous sequences in other Gα proteins. These differences will probably have significant functional consequences. *In situ* hybridization shows that the $G\alpha_a$ gene is specifically expressed in a minority of neurons within the *Lymnaea* central nervous system, suggesting that this atypical G protein subunit has a cell-specific function. Our findings indicate that the G protein family is yet more elaborate, implying that additional members remain to be discovered.

MATERIALS AND METHODS

Animals—L. stagnalis were bred under standard laboratory conditions and fed lettuce *ad libitum*. Adult specimens were used (shell height, 25–35 mm).

PCR[1] *Cloning*—Total RNA was isolated from *Lymnaea* central nervous systems (11) and reverse-transcribed with oligo(dT) as a primer using Moloney murine leukemia virus reverse transcriptase according to the manufacturer (Life Technologies, Inc.). Degenerate PCR primers were based on conserved amino acid motifs DVGGQ and FLNKKD of Gα domains C and G, respectively. The sense primer was as follows: GAL5, 5′-AGA*GAATTC*GA(T/C)GTIGGIGGICA-3′ (*Eco*RI cloning site in italics, I = inosine). Antisense primers included GAL6, 5′-CAC*GGATCC*(A/G)TC(T/C)TT(T/C)TT(A/G)TT(G/A/T/C)AG(A/G)AA-3′ and GAL7, 5′-CAC*GGATCC*(A/G)TC(T/C)TT(T/C)TT(A/G)TT(T/C)AA(A/G)AA-3′ (*Bam*HI cloning site in italics). The antisense primer was synthesized as two separate pools of oligonucleotides to reduce redundancy (12).

PCR was carried out with one-tenth of an animal equivalent of cDNA and 10 μg/ml each of GAL5 and either GAL6 or GAL7 (in 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.1% Triton X-100, 200 μM of each dNTP, 0.2 units of Super *Taq* polymerase (HT Biotechnology); 40 cycles of 1 min at 94 °C, 1 min at 40 °C, and 1

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) Z47551.

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[1] The abbreviation used is: PCR, polymerase chain reaction.

min at 72 °C). Under these conditions, the GAL5-GAL6 combination yielded, among others, a product of an appropriate size (~ 240 base pairs), whereas the GAL5-GAL7 combination did not. The GAL5-GAL6 product was subcloned using standard procedures (13). Inserts were sequenced with vector-specific primers using Sequenase 2.0 according to the manufacturer's instructions (U. S. Biochemical Corp.).

Isolation of cDNA Clones Encoding $G\alpha_a$—A Lymnaea central nervous system-specific cDNA library (constructed in UniZAP XR (Stratagene); 29 subpools of approximately 50,000 independent clones)[2] was screened in a PCR-based procedure (14). Clone-specific primers (GAA1, antisense, 5′-TCATCATACGCGGTCAAGTTGAC-3′; GAA2, sense, 5′-GTCAACTTGACCGCGTATGATGA-3′) were used in combination with vector-specific primers to screen all library subpools. Selected cDNA clones were isolated by screening approximately 75,000 plaques of the pertinent subpool in a PCR-based strategy (15). The R408a helper phage was used in the *in vivo* excision procedure described by the supplier (Stratagene) to recover the cDNA inserts in phagemids.

Characterization of GAAL1 and GAAL2 cDNAs—Both strands of cDNAs were sequenced with Sequenase 2.0, applying a walking primer strategy. Ambiguous regions in the sequence were re-examined by cycle sequencing with Super *Taq* using a SequiTherm kit (Epicentre Technologies) according to the manufacturer's instructions.

In Situ Hybridization—The insert of pGAAL2 was PCR-amplified with vector-specific primers containing T3 and T7 RNA polymerase promoter sequences, respectively. Digoxigenin-labeled cRNA probes were synthesized using a commercial kit (Boehringer Mannheim) and hybridized with 7-μm sections of the *Lymnaea* central nervous system (9).

RESULTS

Identification of a Novel G Protein α Subunit Expressed in Lymnaea—Degenerate oligonucleotides were based on well conserved Gα amino acid motifs within domains C and G of the nucleotide-binding pocket (Ref. 16; see "Materials and Methods"). Using these oligonucleotides as primers, PCR was performed with *Lymnaea* central nervous system-specific cDNA as a template. A PCR product of the appropriate size was subcloned, and the insert of multiple clones was characterized by nucleotide sequence analysis.

In a collection of 34 clones analyzed, we encountered multiple known *Lymnaea* cDNA sequences, coding for $G\alpha_o$ (8 clones), $G\alpha_i$ (5 clones), or $G\alpha_q$ (3 clones). The ratio of these clones is roughly similar to their relative abundance in the *Lymnaea* brain (data not shown). The other 18 clones, however, contained an identical and novel sequence. Upon conceptual translation it appeared to encode (part of) a G protein α subunit, as deduced from the presence of hallmark amino acid motifs directly flanking the residues encoded by the PCR primers. The predicted amino acid sequence was clearly different from that of all *Lymnaea* Gα subunits characterized by us before (8, 10). Moreover, it did not resemble any other Gα sequence described to date.

Isolation and Characterization of cDNA Clones Encoding $G\alpha_a$—To isolate cDNA clones harboring the full protein-coding region for the newly identified Gα subunit, we screened a *Lymnaea* central nervous system-specific cDNA library in a PCR-based strategy (14, 15). At least 25 out of approximately 1.5×10^6 independent clones appeared to be $G\alpha_a$-specific. Two of these were isolated for further analysis and designated GAAL1 (**G**-Alpha **A**jax of ***Lymnaea***) and GAAL2, respectively (Fig. 1).

Partial nucleotide sequence analysis revealed that the GAAL1 insert (3.5 kilobases) corresponds to an immature RNA transcript, as it starts with (part of) a retained intron within the domain A-encoding part (Fig. 2*A*). The 3′-intron-exon boundary follows a sequence of highly repetitive nature and exhibits a sequence (cag/GT) that complies to the consensus for splice acceptor sequences (17). The position of the intron is identical to that of intron 1 in $G\alpha_i$ and $G\alpha_s$ class genes as well as Gα genes of *Caenorhabditis elegans* (18–20). In general, this intron tends to be large. Indeed, as pGAAL1 misses the 5′-part of the intron, the length of the latter should exceed 0.5 kilobases (Fig. 2*A*).

The GAAL2 cDNA does contain a complete open reading frame. Fig. 2*B* shows the nucleotide sequence and conceptual

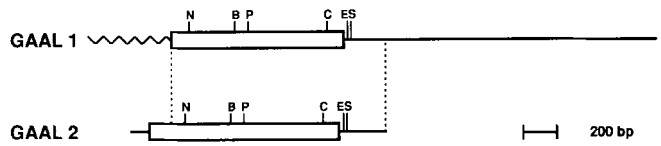

FIG. 1. **Schematic representation and restriction sites of the GAAL1 and GAAL2 cDNAs.** *Open boxes* represent protein-coding sequences; *straight lines* represent untranslated sequences, and the *wavy line* indicates retained intron sequences within the GAAL 1 cDNA. *B*, *Bam*HI; *C*, *Cla*I; *E*, *Eco*RI; *N*, *Nco*I; *P*, *Pst*I; *S*, *Sma*I.

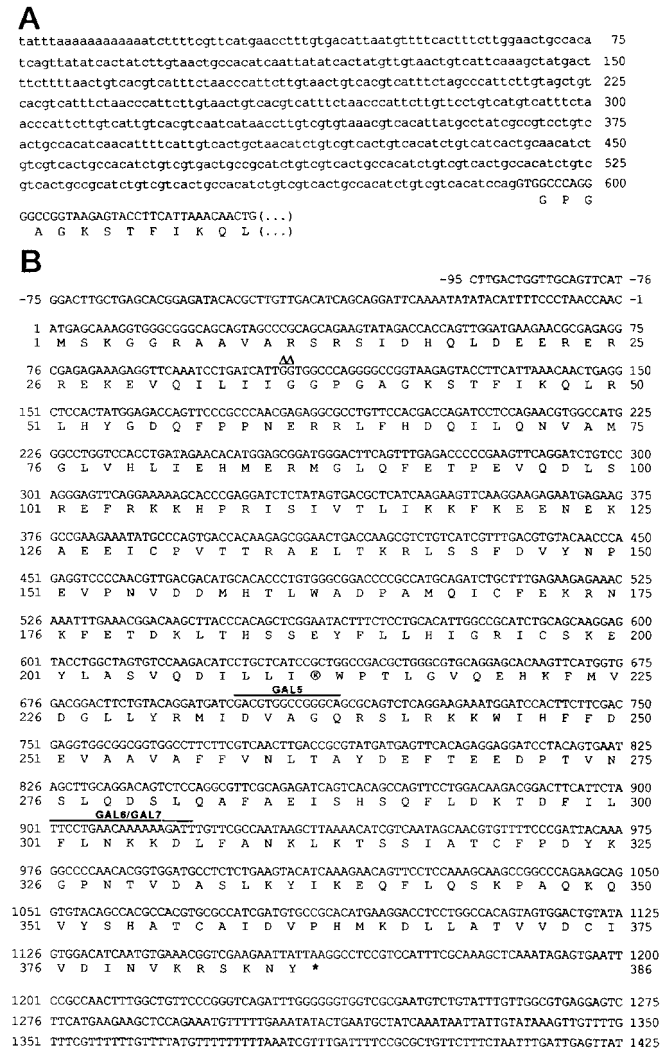

FIG. 2. **Characterization of $G\alpha_a$-encoding cDNAs.** *A*, partial nucleotide sequence of the GAAL1 cDNA. The 5′-part of GAAL1, harboring the downstream part of a putative retained intron and the 3′-intron-exon boundary, was sequenced. Intron sequences are given in *lower case letters*, and exon sequences and the encoded amino acids are given in *upper case letters*. *B*, nucleotide sequence of the GAAL2 cDNA and conceptual translation. The regions that were used to devise degenerate PCR primers GAL5 (sense) and GAL6 or GAL7 (antisense) are *overlined*. *Triangles* indicate the position of the intron in the GAAL1 cDNA, and an *asterisk* denotes the stop codon. The arginine residue that is conserved in all G protein α subunits (Arg-212 in $G\alpha_a$) and serves as a cholera toxin substrate in $G\alpha_s$ and $G\alpha_t$ has been *circled*. Numbering of the nucleotide sequence is relative to the start codon.

[2] E. Vreugdenhil, unpublished data.

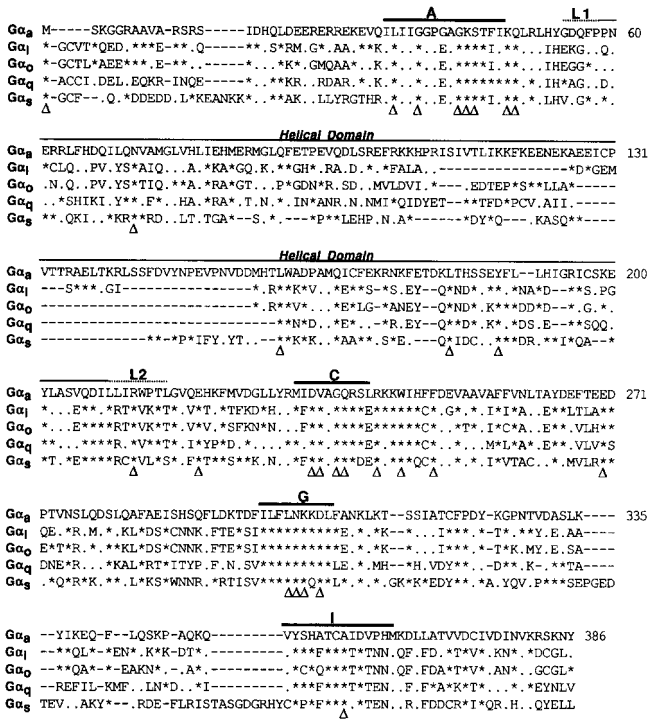

FIG. 3. **Comparison of $G\alpha_a$ with other *Lymnaea* G protein α subunits.** Alignment of the amino acid sequence of the $G\alpha_a$ protein with that of *Lymnaea* $G\alpha_i$, $G\alpha_o$, and $G\alpha_s$ (8) as well as *Lymnaea* $G\alpha_q$ (10) is shown. Numbers are relative to the $G\alpha_a$ sequence. Guanine nucleotide-binding motifs A, C, G, and I (16) are indicated by *thick overlining*. The helical domain (24) has been indicated by *single overlining*, whereas linkers L1 and L2, which connect the helical domain to the rest of the protein, are indicated by *dotted overlining*. *Asterisks* indicate identical amino acid residues, *dots* indicate conserved substitutions, and *dashes* indicate gaps to allow for optimal alignment. Amino acid residues that appear to be conserved in G protein α subunits are indicated by *triangles underneath* the compared sequences.

translation of the 1541-base pair insert. No *bona fide* polyadenylation signal can be found at the 3′-end of GAAL2, which probably arose from internal priming by the oligo(dT) primer used for reverse transcription. Since the GAAL1 cDNA contains approximately 1.9 kilobases of trailer sequences and open reading frame analysis of GAAL2 revealed a putative coding region of 1158 base pairs, the corresponding mRNA could be as long as 3 kilobases provided that alternative splicing does not occur. The GAAL2 cDNA specifies a 386-amino acid protein with a theoretical molecular weight of 44,666. The reading frame starts with a methionine codon in an appropriate sequence context for translation initiation (21). Within the deduced protein sequence, domains A, C, G, and I of the putative guanine nucleotide-pocket can be easily discerned (Ref. 16; see also Fig. 3). The novel G protein α subunit was designated $G\alpha_a$.

Comparison of $G\alpha_a$ with Other Gα Subunits—Fig. 3 shows an alignment of the predicted amino acid sequence of $G\alpha_a$ with that of previously described *Lymnaea* G protein α subunits (8, 10). The primary structure of $G\alpha_a$ turns out to be only slightly similar to these proteins. In fact, it exhibits a low degree of amino acid sequence identity (27–33%) with any Gα subtype described to date, ranging from vertebrate and invertebrate members of the $G\alpha_i$, $G\alpha_s$, $G\alpha_q$, and $G\alpha_{12}$ subclasses to putatively species-specific subunits like $G\alpha_f$ and concertina of *Drosophila* (22, 23) and the GPA proteins of *C. elegans* (20). In sharp contrast, previously identified *Lymnaea* Gα proteins share a very high degree of sequence identity with their respective vertebrate and invertebrate counterparts (76–90%; Refs. 8 and 10). Amino acid identity percentages such as observed for $G\alpha_a$ generally indicate that the compared proteins are members of different Gα subclasses. Therefore, we consider $G\alpha_a$ to represent a novel subtype, defining a distinct class of G protein α subunits by itself.

$G\alpha_a$ is most distinct in the so-called helical domain (24), encompassing amino acids 54–218. This domain is the most heterogenous part of G protein α subunits and includes a region of structural disparity between $G\alpha_t$ and $G\alpha_i1$, which might be related to their interaction with different proteins (25). The $G\alpha_a$ helical domain appears to bear an insert when compared with other Gα proteins (Fig. 3).

Different as $G\alpha_a$ may be, it does contain 41 out of 63 residues that are conserved between members of the $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$ classes (not shown). When taking into account the Gα subunits of the slime mold *Dictyostelium* (26) and the $G\alpha_s$ protein of the trematode *Schistosoma mansoni* (27), 27 residues obey a new consensus of 31 invariant amino acids (indicated by *triangles* in Fig. 3). Thus, $G\alpha_a$ harbors virtually all residues which, as deduced from their strict conservation, appear to play pivotal roles in Gα function.

Guanine Nucleotide Binding Motifs—Intriguingly, the sequence of the A domain of the putative guanine nucleotide binding pocket of $G\alpha_a$ is significantly different from the consensus sequence LLLLG(A/T/P)(G/S)(E/N)SGK that can be deduced from a compilation of G protein α subunits reported thus far. Nonetheless, it complies to the minimal consensus for this domain (G*XXXX*GK) as deduced from a variety of nucleotide-binding proteins (28). The $G\alpha_a$ C domain is also distinct, deviating from the highly conserved sequence in other Gα proteins. First, the DVGGQR motif has been changed into DVAGQR (difference underlined), again leaving unaffected the minimal consensus sequence (D*XX*GQ; Ref. 28). Second, immediately downstream of this motif, a highly conserved glutamic acid residue has been replaced by leucine in the sequence SLRKKWIH. The unique features of the A and C domains of $G\alpha_a$ described above add to our knowledge concerning these crucial elements of Gα proteins (see "Discussion"). The G and I domains of $G\alpha_a$ are identical and similar, respectively, to analogous regions in other G protein α subunits.

Post-translational Modification Sites—Although not an absolute prerequisite for all Gα proteins, N-terminal myristoylation of various G_i class α subunits is supposed to enhance interaction with the Gβγ complex and the plasma membrane (29). The $G\alpha_a$ N terminus lacks the MG*XXX*(S/T) consensus required for myristoylation. In addition, it does not contain a cysteine residue that could be palmitoylated (30, 31) or modified by attachment of arachidonic acid (32) as another way of creating a hydrophobic membrane anchor. Consequently, $G\alpha_a$ is either soluble upon dissociation of the heterotrimer or it uses other means to attach to the inner face of the plasma membrane.

The arginine residue that is conserved in all G protein α subunits and serves as a substrate for ADP-ribosylation by cholera toxin in $G\alpha_s$ and $G\alpha_t$ (1) is also present in $G\alpha_a$. Solely on the basis of the primary structure of $G\alpha_a$, it cannot be concluded, however, whether the protein can actually be modified by the toxin. Another bacterial toxin, pertussis toxin, modifies some G_i class members through ADP-ribosylation of a cysteine residue four residues away from the C terminus (1). Since $G\alpha_a$ lacks a cysteine at this position, the G_a protein will subserve pertussis toxin-insensitive functions.

Spatial Expression Profile of $G\alpha_a$—To examine the expression of $G\alpha_a$ within the *Lymnaea* central nervous system, we performed *in situ* hybridization experiments (Fig. 4). Synthetic antisense RNA derived from the GAAL2 cDNA was used as a probe. Interestingly, the corresponding mRNA appears to be expressed in a highly cell-specific fashion in that the probe

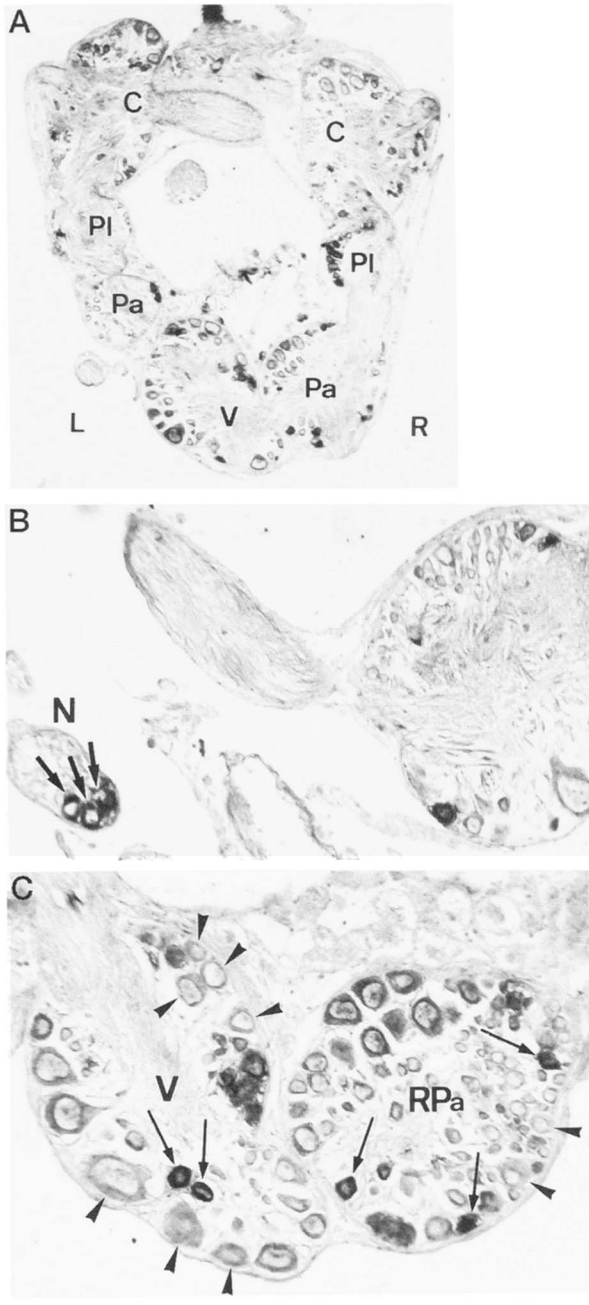

FIG. 4. ***In situ* hybridization of the *Lymnaea* central nervous system with a GAAL2-specific probe.** Sections of the *Lymnaea* brain were incubated with a cRNA probe prepared from the complete GAAL2 cDNA. *A*, overview. *C*, cerebral ganglia; *Pl*, pleural ganglia; *Pa*, parietal ganglia; *V*, visceral ganglion; *R*, right; *L*, left. The pedal ganglia are not present in the section shown. *B*, detail of a visceral ganglion. *Arrows* indicate yellow cells within a sectioned nerve (*N*). *C*, detail of visceral (*V*) and right parietal (*RPa*) ganglia. *Arrows* indicate neurons that appear to highly express $G\alpha_a$-specific mRNA; *arrow heads* indicate cells that do not hybridize with the GAAL2 cRNA above background.

hybridizes to a limited number of neurons (less than 10%) dispersed throughout the brain (Fig. 4*A*). For example, $G\alpha_a$ mRNA is expressed in the so-called yellow cells, which are located, among others, in the visceral, parietal, and pleural ganglia, but also in the nerves originating from the visceral ganglia (Fig. 4*B*; for review see Ref. 33). Other well studied neurons, however, like the light green cells and the caudodorsal cells in the cerebral ganglia (6) do not express $G\alpha_a$ at a detectable level (not shown). As another example, Fig. 4*C* shows a section of the visceral and right parietal ganglia in which cells that express $G\alpha_a$-encoding transcripts at a high level abut cells that do not. These data suggest that the $G\alpha_a$ protein fulfils cell type-specific functions.

DISCUSSION

In this paper, we describe a novel G protein α subunit, $G\alpha_a$, which is expressed differentially in the central nervous system of the pond snail, *L. stagnalis*. We feel confident that we have indeed cloned the α subunit of a heterotrimeric G protein rather than a member of the Ras family of small guanine nucleotide-binding proteins. First, its putative nucleotide-binding domains resemble those of Gα proteins much more than those of the Ras family. This also holds true for the sequence directly following domain C, which constitutes a major conformational switch domain of G protein α subunits (34). Second, its molecular mass (44.7 kDa) is within the range of Gα proteins (39–45 kDa) but differs significantly from that of the Ras family (21 kDa). Third, $G\alpha_a$ contains many amino acids that appear to be invariant in G protein α subunits. Among these is the strictly conserved arginine residue (35) (Arg-212 in $G\alpha_a$) that serves as a cholera toxin substrate in $G\alpha_s$ and $G\alpha_t$ (1) and appears to play a key role in stabilizing the transition state during GTP hydrolysis (25, 36). $G\alpha_a$ also contains the conserved threonine and glycine residues (Thr-217 and Gly-237), which initiate the conformational changes that occur upon guanine nucleotide exchange (34), and the crucial glutamic acid (Glu-270) that propagates these changes to other parts in the molecule. The glutamine residue (Gln-238) that is supposed to fulfill a key role during GTP hydrolysis (25, 36) has also been preserved. Amino acid sequence comparison indicates that $G\alpha_a$ resembles other Gα subtypes described to date (27–33% identity) but clearly defines a distinct subclass.

Of the nucleotide-binding motifs, the A domain is involved in binding phosphate groups (24, 25). Although the core sequence of this domain in $G\alpha_a$ (GGPGAGK) differs considerably from known Gα A domains, it fits the G(G/P/A)PG*X*GK consensus for another group of nucleotide-binding proteins, the adenylate kinases (37). Nonetheless, Gα subunits with A domain sequences differing from the formerly canonical GAGESGK motif exhibit deviating nucleotide affinities and kinetics of GTP hydrolysis (38, 39). $G\alpha_a$ may therefore exhibit a GTPase cycle with greatly distinct features.

The C domain of G protein α subunits coincides by and large with an important conformational switch element (switch II) (34) and is highly conserved. Surprisingly, $G\alpha_a$ violates the consensus sequence (DV<u>A</u>GQR instead of DV<u>G</u>GQR). Such a deviation is also present in the Gα6 and Gα7 subunits of *Dictyostelium* (26). The DVGGQR motif appears to propagate GTP-induced conformational changes to the switch II region (24, 34). It has been suggested that the two glycines of the motif provide the necessary freedom for this action (24). Indeed, the latter is hampered by mutation of the second glycine to alanine (40). It is, however, highly unlikely that substitution of alanine for the first glycine has similar effects, since it has now been found to occur naturally in three independent Gα subunits. Moreover, the strict conservation of alanine in an analogous position in Ras(-like) proteins, and the fact that mutations in this position are implicated in cellular transformation (41), point to a functional significance of the presence of such a residue in *Lymnaea* $G\alpha_a$.

Another salient deviation of $G\alpha_a$ is found immediately downstream of the DVAGQR motif. Hitherto, the glutamic acid in the sequence S<u>E</u>RKKWIH appeared to be strictly conserved among all Gα subunits. On the basis of crystal structure studies, it was predicted that this residue acts as a general base during GTP hydrolysis, activating a water molecule for a nucleophilic attack on the γ-phosphate group (24). However, $G\alpha_a$ has leucine at this position, and it is very difficult to imagine

that such a residue could perform a similar task. Indeed, a mutational study of the importance of the pertinent glutamic acid refutes the hypothesis (43). Moreover, reports on the structure of GDP·AlF4$^-$-bound Gα subunits (mimicking the transition state) (25, 36) support the notion that it is the conserved DVXGQR glutamine that plays an important role (35).

Overall, the helical domain is the most heterogenous part of Gα proteins. It is thought that this domain constitutes an independent, built-in GTPase-activating domain (35, 42). In addition, the helical domain acts as an intrinsic guanine nucleotide dissociation inhibitor by burying the guanine nucleotide-binding pocket (24). Its unique amino sequence in $G\alpha_a$ suggests that there are few constraints on its (primary) structure. Such a sequence diversity might be exploited for subtype-specific interactions with other proteins.

The functions of $G\alpha_a$ are beyond speculation as yet but are likely to involve transmembrane signaling. It will be very interesting to elucidate which receptors and effectors are coupled by this Gα subtype. Biochemical and mutational studies of $G\alpha_t$ and $G\alpha_q$ have implicated the extreme C terminus, the equivalents of $G\alpha_a$ residues 347–364, and possibly the N terminus in receptor coupling (44, 45). The corresponding regions of $G\alpha_a$ do not resemble their counterparts in any other Gα protein. A similar conclusion may be drawn with respect to the effector-interaction interface. Effectors of $G\alpha_s$ (adenylyl cyclase) and $G\alpha_t$ (cGMP-specific phosphodiesterase) interact with discrete regions on one face of the α subunits (46–48). These regions encompass their α2-β4, α3-β5, and α4-β6 parts (24, 34), corresponding to $G\alpha_a$ residues 245–251, 270–297, and 330–350, respectively. $G\alpha_a$ is significantly dissimilar in the latter two regions. Thus, although our understanding of the Gα domains that interact with receptors and effectors is far from complete, it appears that $G\alpha_a$ would functionally link other receptors and effectors than hitherto described Gα subtypes. Although transmembrane signaling is an obvious possibility in terms of $G\alpha_a$ function, it cannot be excluded that $G\alpha_a$ is involved in other processes, like intracellular trafficking (49–51).

Irrespective of the precise nature of its tasks, $G\alpha_a$ appears to subserve cell-specific functions. $G\alpha_a$-specific mRNA is specifically expressed in a restricted set of cell types within the *Lymnaea* brain. Among these are the yellow cells, which are considered to be involved in osmoregulation (52). Yet, at this stage it is hard to relate $G\alpha_a$ expression in these cells to specific function(s). In view of the apparent cell type specificity, it will be instructing to know whether or not $G\alpha_a$ expression is specifically restricted to the central nervous system. We also need to assess whether $G\alpha_a$ is *Lymnaea*- or mollusc-specific or whether similar proteins exist in higher organisms like vertebrates. It will be very interesting to express the $G\alpha_a$ protein and determine its biochemical properties and to perturb its function with antisense DNA or antibodies and assess its cellular role(s).

$G\alpha_a$ is the fifth *Lymnaea* G protein α subunit identified by our group. Even the unicellular slime mold, *Dictyostelium discoideum*, has a Gα family consisting of at least 8 members (26). In *Drosophila*, atypical G protein α subunits like concertina and $G\alpha_f$ have been discovered (22, 23). Since the PCR might very well miss certain Gα subtypes, especially when these subunits harbor a different sequence in the parts that were used to develop degenerate primers, we surmise that a range of *Lymnaea* Gα proteins might have escaped detection. The identification of $G\alpha_a$, a Gα subunit with clearly distinct properties, indicates that the ever increasing array of Gα subtypes has not yet met its limit.

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